SYNOPSIS OF THE THESIS TO BE SUBMITTED

TO THE UNIVERSITY OF NMIMS (DEEMED-TO-BE UNIVERSITY)

FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY (CHEMISTRY)

NAME OF THE CANDIDATE	**Mr. Amit Vishwanath Pawar**
DOCTORAL COMMITTEE	**Dr. Evans Coutinho,** Professor at Bombay College of Pharmacy, Mumbai **Mr. Ajay Goyal,** Sr. Scientist, Analytical Development, Johnson & Johnson Pvt Ltd, Mumbai
NAME AND DESIGNATION OF MENTOR /CO-MENTOR	**Dr. Nancy Pandita**, Former Professor and Head of Chemistry Department, Sunandan Divatia School of Science, SVKM's NMIMS University (deemed-to-be), Mumbai-400056. **Dr. Nitin Hardas,** Former Associate Director, Analytical Development, Johnson & Johnson Pvt Ltd, Mumbai
TITLES OF THE THESIS	Analytical Method Development of Stability indicating UPLC methods for Antipsychotic drugs by "QbD" approach
PLACE OF RESEARCH	Sunandan Divatia School of Science, SVKM's NMIMS University (deemed-to-be), Vile Parle, (W), Mumbai-400056.
NUMBER AND DATE OF REGISTRATION	75121150003 / 03-Dec-2016
DATE OF SUBMISSION OF SYNOPSIS	
SIGNATURE OF CANDIDATE	Amit Pawar
SIGNATURE OF MENTOR	Nancy Pandita
SIGNATURE OF CO-MENTOR	N. R. Hardas

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Abstract:

Analytical testing becomes increasingly important in pharmaceutical development including risk ass essment, process monitoring, control, and continuous quality checks since there is a large increase in the population and new diseases.

If proper scientific studies are not conducted during development phase, it may lead to failure or investigation during stability studies. Method Development strategy plays an important role in quality and cost of the product. Modern approaches like QbD (Quality by Design) make it possible to develop simple, cost effective and robust methods. QAbD is a systematic process used to develop products starting with pre-defined objectives, with an emphasis on understanding the products and controlling them professionally with solid science. Significance of method developed by QbD approach is, as per the FDA guidance working within the Method Operable Design Region which is also known as design space is not considered as a change. Movement out of the design space is considered to be a change and would normally initiate a regulatory post approval change.

QbD method development approach is aligned with the upcoming USP <1220> and ICH Q14 guidelines of life cycle management of analytical methods. Based on risk and science-based information, we developed UPLC (Ultra Performance Liquid Chromatography) methods for antipsychotic drugs are developed by applying a Quality by Design approach. A systematic review of all key components of the RP*UHPLC methods was carried out during development in order to create a robust experimental design. Therefore, the developed methods are simple, accurate and precise for both active compounds and degradation compounds. While developing method “Platforming approach” was used, which means same method can be used for drug substance as well as for drug products. Method developed for Haloperidol drug can separate 12 impurity peaks and one active peak in 21 minutes run time. Same method can be employed for Haloperidol tablets formulation as well. Method developed for Risperidone drug can separate total 10 peaks in 12 minutes run time. Platforming approach is having implausible advantage in R & D and stability labs to perform comparison and trending of impurity profile in drug substance and drug products throughout the life cycle of product.

Developed methods are validated according to ICH Q2 (R1) & ANVISA guidelines (RDC N0 166, Dated July 24th, 2017).

By using methods developed by this approach, a laboratory can improve the performance of methods, reduce analytical uncertainties such as out-of-specification (OOS) and out-of-trend (OOT) results. These developed methods can be alternative to Pharmacopeial methods and can be used for routine analysis in quality control laboratories.

Key words: Haloperidol, Risperidone, QbD , MODR , UHPLC , ICH Q14, USP <1220>, Design space

Chapter 1: Introduction & Literature review

1.1 INTRODUCTION

Increase in the population and new diseases there is a huge and continuous demand for the discovery of new drugs and continuous research in currently available drugs. “Fast to Market” availability of drugs is subjected to safety and efficacy of the drugs. Analytical methods play an important role in this area. Results generated using analytical procedures provide the basis for key decisions regarding compliance with regulatory, compendial, and manufacturing limits for the drugs. Precise, specific, and robust nature of any analytical method is depending upon the way analytical method is developed. OFAT (one factor at a time) is the typical approach for chromatographic analytical method development work which normally begins with change in one variable at a time to get a suitable method. Due to this combined effect of variables remains unexplored and creates major risk of failures in terms of robustness of method. A major risk of using OFAT approach is that this provide extremely limited coverage of an experimental region encompassing a potential final design space. Additionally, method development by using OFAT approach take lot of time [1]. Number of guidelines are available for the validation of the analytical procedures but after understanding the need of the robust methods ICH, as well as the United States Pharmacopeia coming up with the enrollment of new guidelines that includes Life cycle management of analytical procedures and need of Quality by Design approach in analytical method development [2]. For controlling analytical procedure throughout its lifecycle, a three-stage concept was proposed by USP which includes method design, method Qualification and finally continuous method verification to ensure that the method remains in a state of control during routine use. This will help to develop the analytical methods fit for its intended purpose in short time and can be useful to consider the entire lifecycle of an analytical procedure.

Quality by design is more comprehensive, scientific, systematic, and risk-based strategy approach for the development of product or analytical methods. In this approach multiple parameters and different setting are explored which supports to develop broad knowledge about the impact of factors on the method performance. Based on this knowledge of impact of multidimensional combinations of variables and verification of parameter region meeting method performance criteria method operable design region (MODR) or design space is get established. As per the FDA guidance working within the design space is not considered as a change. Movement out of the design space is a change and would normally initiate a regulatory post approval change process [3, 4]. Proper implementation of QbD can potentially provide three main benefits for development: A) More

efficient use of development time and costs, B) Ability to meet FDA submission guidelines and expectations, C) Reduced approval times – and fewer queries – from the FDA. Additionally, by using methods developed by this approach, a laboratory can improve the performance of methods, reduce analytical uncertainties such as out-of-specification (OOS) and out-of-trend (OOT) results. In pharma industry HPLC (High performance Liquid chromatography) is most widely used for Assay, Purity and CU test analysis for different kind of dosage forms. HPLC throughput was limiting efficiency. Laboratories using HPLC couldn't keep pace with the urgency of business demands to analyze more samples, faster, and with better results. By using modern techniques like UHPLC (Ultra High-Performance Liquid Chromatography) we can reduce the analysis time for the drug/ drug products which will eventually help in reducing the cost of the product. Additionally, if a single LC-MS compatible, stability indicating chromatographic method for testing assay, Purity of drug substance and drug product with shorter run time, developed by using QbD approach will have added advantage for the labs.

The drug molecules selected for the study belong to the class of antipsychotic drugs. Antipsychotic drugs are used as a short or long-term treatments for bipolar disorder to control psychotic symptoms such as hallucinations, delusions, or mania symptoms. These symptoms may occur during acute mania or severe depression. Some also treat bipolar depression, and several have demonstrated long-term value in preventing future episodes of mania or depression.

1.2 REVIEW OF LITERATURE

Antipsychotic drugs classified into two different types of different types a) Typical antipsychotics and b) atypical antipsychotics. Typical antipsychotics or the first-generation antipsychotics are the drugs developed before 1950 e.g. (Haloperidol, Thorazine). Atypical antipsychotics are the drugs approved in 1990. E.g., Clozapine, Risperidone, Paliperidone. Antipsychotic drugs work by altering the effect of certain chemicals in the brain, called dopamine, serotonin, noradrenaline, and acetylcholine. These chemicals have the effect of changing human behavior, mood and emotions. Dopamine is the main chemical that these medicines have an effect on. By altering the effects of these chemicals in the brain they can suppress or prevent person from experiencing: Hallucinations (such as hearing voices), Delusions (having ideas not based on reality), Thought disorder, Extreme mood swings that are associated with bipolar disorder.

In the present work, two drug molecules, Haloperidol and Risperidone and their formulations were selected for the study. There are analytical methods reported for determination of active and related impurities by HPLC. Many methods available in the different pharmacopeia and various literature.

Comparison of different Pharmacopeial methods:

Product	Test	Pharmacopeia References			
		USP	EP	JP	Ch.P
Haloperidol DS	Assay	√ -Non aqueous Titration	√ -Non aqueous Titration	√ -Non aqueous Titration	√ -Non aqueous Titration
	Purity	√	√	√	√
Haloperidol Tablet	Assay	√	XX	√	√ -By UV
	Purity	x	XX	x	√
Risperidone DS	Assay	√	√ -Non aqueous Titration	√	XX
	Purity	√	√	√	XX
Risperidone Tablets	Assay	√	XX	√√	XX
	Purity	√	XX	√√	XX
Risperidone Oral solution	Assay	√	XX	√√	XX
	Purity	√	XX	√√	XX

LC-MS Compatible method	√ - Product and test available	√√ - Combined Assay/Purity test
LC-MS Incompatible mehods	X -Test not available	XX- Product not available

For both the molecule's reported methods are mainly based on the High-Performance liquid chromatography [5-7]. Some methods are also available on UHPLC [8, 9]. Some methods are reported with the use of QbD approach for development of analytical method [10] but very few methods are mass compatible and common for assay and purity. Literature review does not reveal any QbD based short run time UHPLC combined assay, purity method with platforming approach which can be used for drug substance as well as for different formulations. Limited literature available for the combine methods for assay of actives and related substances methods for drug substance as well as drug product. Information about the related impurities of Haloperidol and Risperidone is available in many articles as well as in Pharmacopeias [11] [12, 13]. No reference found for the method which are developed by using QbD approach, with shorter run time, mass compatible, combined method for assay/Purity which can be used for drug substances as well as related drug products (Formulations). Present work describes the stepwise approach for chromatographic method development by using Quality by design approach. Aim was to develop Stability indicating, mass compatible, shorter run time chromatographic method which can be used to check assay and purity of drug substance as well as drug products. In additional method was validated as per current regulatory requirements. Method

validation of optimized method was performed according to ICH Q2 (R1) [14] & ANVISA guidelines (RDC N0 166, Dated July 24th, 2017)[15].

Chapter 2: Rationale, Aim and Objective

2.1 Rationale

Antipsychotic drugs are very important for the treatment of treat psychosis and other mental and emotional conditions. The global antipsychotic drugs market size was valued at $12,417.60 million in 2020, and is estimated to reach $26,041.00 million by 2030, growing at a CAGR of 8.0% from 2021 to 2030. To support this need and bring the innovative products fast to market analytical methods plays an important role. Apart from developing method, performance of the methods throughout its life cycle is an important aspect. Many times, labs face issue related to failure of transfers, failures for precision which are most of the time related to method performance issue. Hence, the work is focused on developing a combine, shorter run time test method for determination of assay and related substances of active in drug substance as well as drug product. Same method can be used for drug substance as well as multiple drug product formulations. Additionally, to develop robust method QbD approach will be used so that multiple parameters and different setting are explored which supports to develop broad knowledge about the impact of factors on the method performance. Method develop by QbD approach will be more compliant and it will reduce approval times – and fewer queries – from the FDA. Selection of mobile phase buffers was done in such a way that method can be used directly on LC-MS so that

Quality control lab can directly use these methods for LC-MS investigations in case of any unknowns were observed during stability testing. Method developed by using UHPLC technique with will save analysis time, usage of solvents and generate very low waste which makes method environment friendly as low amount of solvent waste will be generated.

2.2 Aim

To Develop and Validate Stability indicating UPLC methods for active and degradation products of Antipsychotic drugs by QbD (Quality by Design) approach

2.3 Objectives

- To perform FD study to check possible degradation conditions/Possible degradants of selected antipsychotic drug
- To develop stability indicating analytical UPLC methods by using QbD approach.
- To challenge the robustness of the method using DoE tool (Design of Experiment).

- ❖ To perform validation of stability indicating methods as per ICH and ANVISA guidelines

Chapter 3: Method development and Validation

3.1 Materials

3.1.1 Risperidone:

In house materials used for Risperidone API, reference material, Imp-A, Imp-B, Imp-C, Imp-D, Imp-E, Imp-F, Imp G, Imp H. Acetonitrile (HPLC grade), methanol (HPLC grade), Glacial acetic acid (AR grade) and ammonia solution 25% (AR grade) were purchased from Merck Germany. Ammonium acetate (HPLC grade, Purity > 99.0%, Purity > 99.0%) and Trifluoro acetic acid (HPLC grade, Purity > 99.0%) were purchased from Sigma Aldrich. HPLC grade water was obtained from Milli-Q water purification system (Millipore, Milford, USA)

3.1.2 Haloperidol:

In house materials used for Haloperidol API, reference material, Imp-A, Imp-B, Imp-C, Imp-D, Imp-E & Imp-F. Haloperidol 1 mg tablets and placebo was obtained from Recipharm, Portugal. Acetonitrile (HPLC grade), methanol (HPLC grade), Glacial acetic acid (AR grade) and ammonia solution 25% (AR grade) were purchased from Merck Germany. Ammonium acetate (HPLC grade, Purity > 99.0%, Purity > 99.0%) and Trifluoro acetic acid (HPLC grade, Purity > 99.0%) were purchased from Sigma Aldrich. HPLC grade water was obtained from Milli-Q water purification system (Millipore, Milford, USA).

3.2 Instruments & Equipment's:

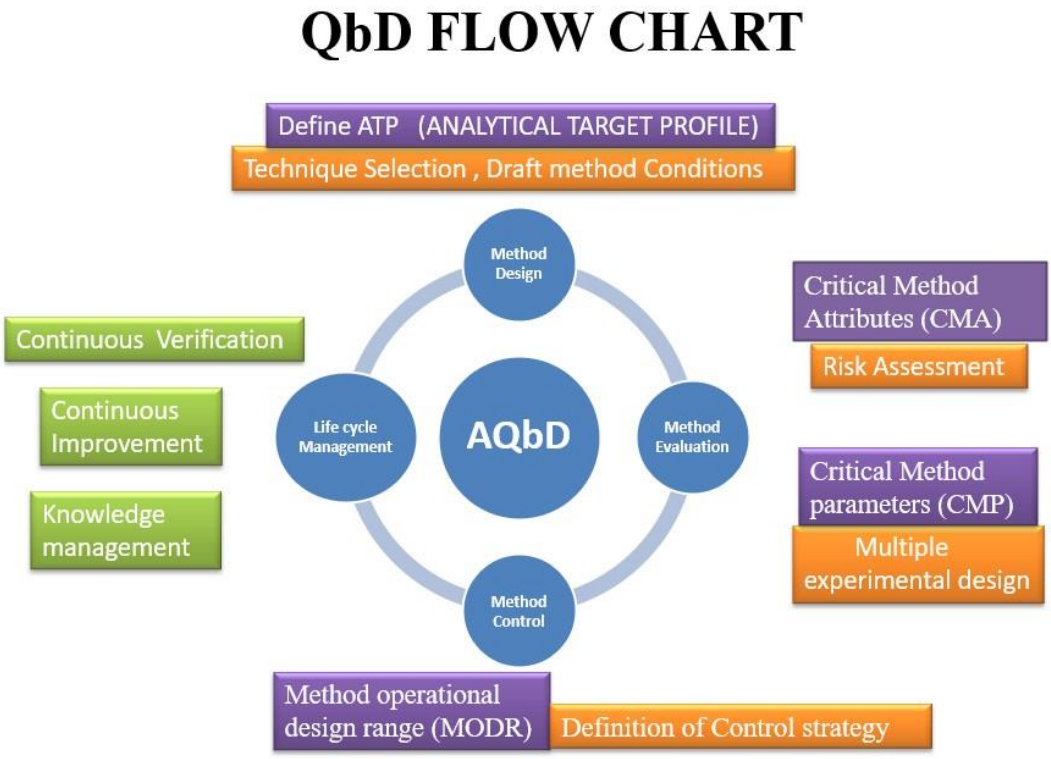
ACQUITY H-class UPLC consisting of Quaternary solvent manager system, Sample manager, Column manager with heater, PDA and EMPOWER 3 software from Waters was used for screening experiments. ACQUITY H-class 2D UPLC consisting of Quaternary solvent manager system, Sample Manager, Column manager with heater, PDA & QDa (Quantum Dalta Analyzer) detector was used for validation. Fusion software (version 9.7.1) by S-matrix was used for designing experiments for method development by QbD approach and Robustness study. Other equipment's used are Oven (Make-Binder), Ultra Sonicator bath (Make- Oscar Ultrasonic).

3.3 Quality by Design:

3.3.1 Quality by design flow:

DOE approach, is very well known as a structured, organized, stepwise approach to study the relationship between different factors. Basic steps involved in any kind of DOE are a) set objective (ATP: Analytical Target Profile), b) brainstorm to select process variables (Risk assessment) or CMA: Critical method Attributes, c) Select an experimental design to study CMV: Critical Method variables) or CMP (Critical method parameters, d) Execute the design e) Analyze the data and interpret the results f) verify predicted results [16].

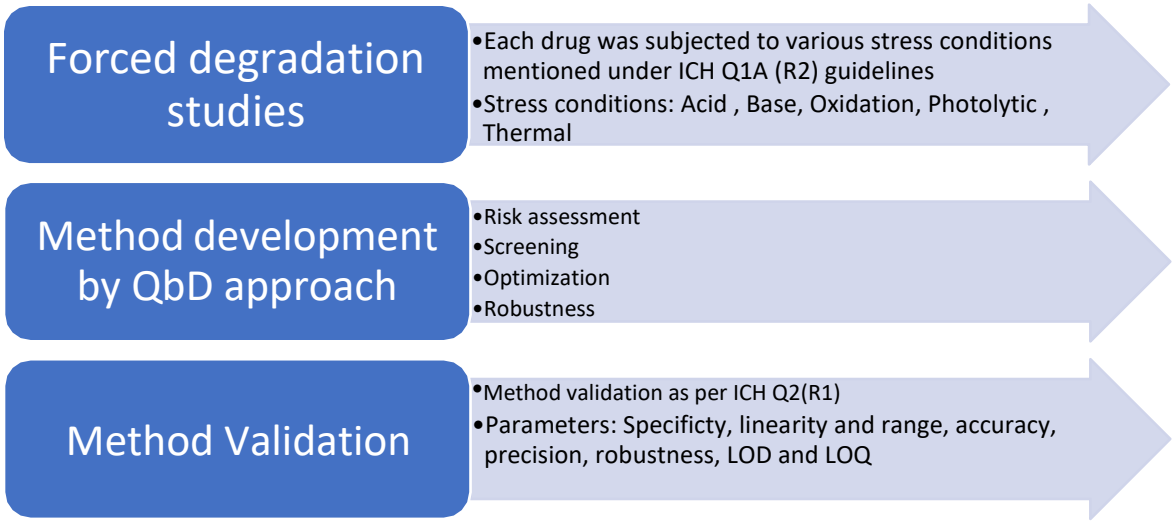
Figure 1 : QbD flow chart



3.3.2: Steps followed during QbD method development

To achieve expected ATP and CMA's, activities were carried into following steps

Figure 2 : Steps followed during method development by QbD approach


Forced degradation studies
•Each drug was subjected to various stress conditions mentioned under ICH Q1A (R2) guidelines
•Stress conditions: Acid , Base, Oxidation, Photolytic , Thermal
Method development by QbD approach
•Risk assessment
•Screening
•Optimization
•Robustness
Method Validation
•Method validation as per ICH Q2(R1)
•Parameters: Specificty, linearity and range, accuracy, precision, robustness, LOD and LOQ

3.3.3: Different conditions for forced degradation studies.

Following are the different conditions which can be used to study the stress testing of any compound[16]. In case of DOE study, it is very much important to know the composition of sample. Composition of sample means number of possible peaks preset in the samples. FDS study will give the tentative idea about the possible peaks which can be present in the sample during the shelf life of product.

Table 1 : Forced degradation conditions

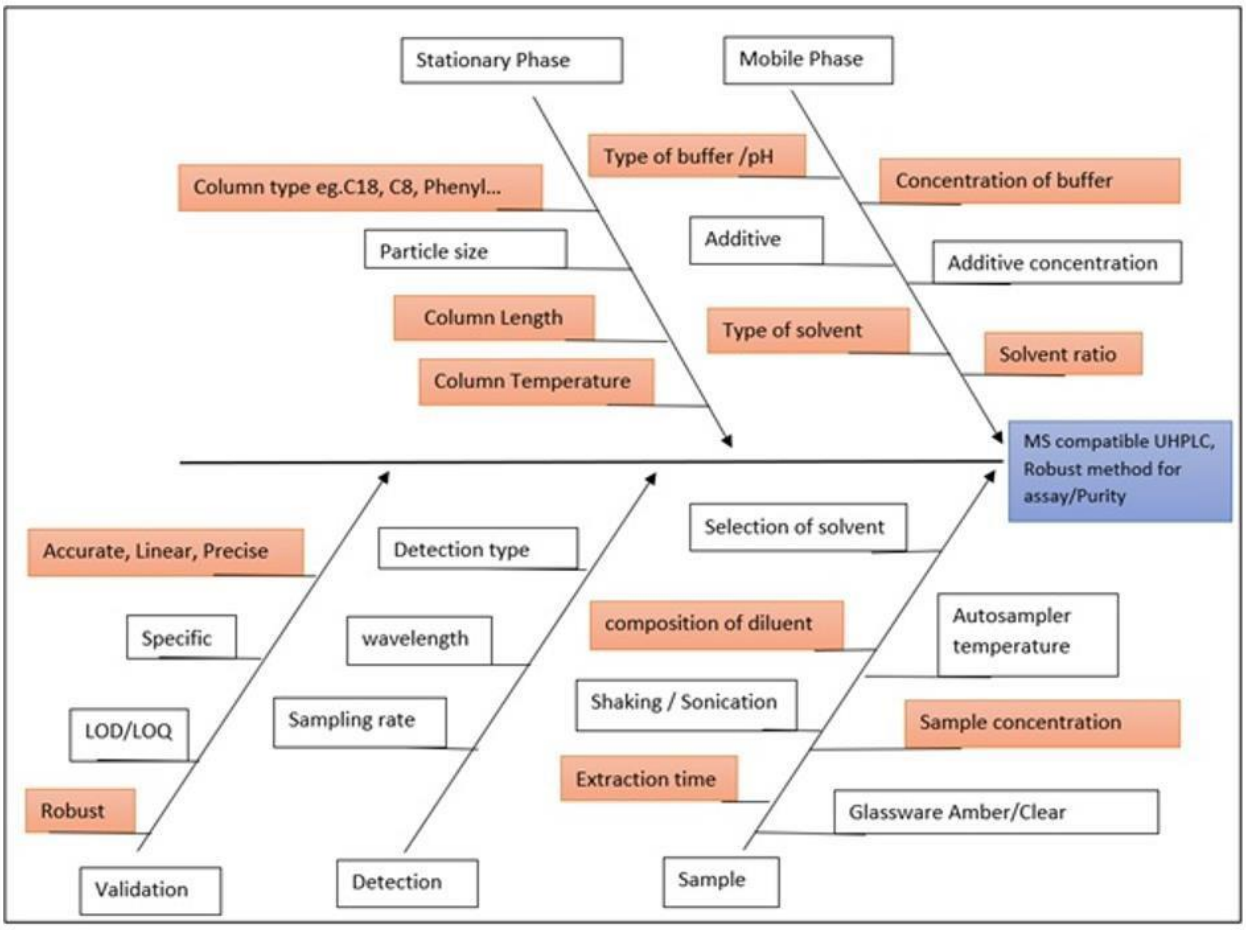
Degradation type	**Experimental condition**	**Storage condition**	**Time Point**
Acid Hydrolysis	0.1 to 5 N HCl	40°C to 70°C	1, 3 ,7 Days
Base Hydrolysis	0.1 to 5 N NaOH	40°C to 70°C	1, 3 ,7 Days
Oxidative	0.3 % to 30% H2O2	Ambient	1, 3 ,7 Days
Photolytic	1 x ICH light, 2 x ICH light	Ambient	1 x ICH light, 2 x ICH light
Thermal	40°C to 100°C	40°C to 100°C	7, 14 days

3.3.4: Method development by QbD approach

3.3.4.1 Risk assessment

Different risk assessment tools can be used to assess the risks associated with the input variables, sample variation and environmental conditions [17].

Figure 3 : Risk assessment perform using Ishikawa diagram to identify the factors which can affect the performance of UHPLC method.



3.3.4.2 Screening & Optimization

For the present study, UHPLC method with UV detection was chosen as the analytical technique and ATP (Analytical target Profile), which defines the intended purpose of the method is set as Method should be: 1) able to separate analyte and impurities from each other, 2) able to quantify the active and impurities accurately and precisely, 3) Mass spectrometry (MS)-compatible mobile phase, 4) statistically challenged validation. To recognize possible aspects that may affect the method performance, structured risk assessment was performed by using Ishikawa diagram (Figure- 1). This diagram shows the critical method parameters (CMP's) which can affect the method performance by impacting critical method attributes (CMAs) like a) Resolution between the peaks should be ≥ 1.5 b) Peak tailing of analyte should be ≤ 2.0, c) Theoretical plates for active peak >10,000.

Table 2 : ATP, CMAs, and CMP considerations for QbD method development

Analytical Method	Assay & Related substances
Analytical target profile (ATP)	• Accurate quantitation of Active substance and Impurities • Stability Indicating • Shorter runtime • Stability indicating method • Common method • Adequate Resolution
Critical Method Attributes (CMA)	• Peak Tailing • Recovery • Peak Purity • Resolution between critical pair of peaks
Critical Method parameters (CMP)	• Flow, • Mobile phase pH • Column temperature • Diluent composition • Sonication time.
Method operational design range (MODR)	• Mobile phase pH ±0.2 (If applicable), • Flow rate ±10%. • Diluent composition range. • Column temperature • Gradient composition

A -Variable selected for Study

- Different column chemistries (6 different Chemistries)
- Different pH
- Different organic solvent / Composition of organic solvent

B - Column Chemistry:

UPLC Column	Dimension	Maximum pH limit
Acquity UPLC HSS T3 C18	150 mm x 1.7 µ , 2.1 mm	2.0-8.0
Acquity UPLC BEH C18	150 mm x 1.7 µ , 2.1 mm	2.0-14.0
Acquity UPLC BEH Shield C18	150 mm x 1.7 µ , 2.1 mm	2.0-14.0
Acquity UPLC CSH C18	150 mm x 1.7 µ , 2.1 mm	2.0-14.0
Acquity UPLC BEH Phenyl	150 mm x 1.7 µ , 2.1 mm	2.0-8.0
Acquity UPLC CSH Fluorophenyl	150 mm x 1.7 µ , 2.1 mm	2.0-14.0

C- Buffer pH:

Buffer	pH
10 mM Ammonium acetate + 0.1% TFA	~ 2.5
10 mM Ammonium acetate + 0.05% Glacial acetic acid	~ 4.5-4.8
10 mM Ammonium acetate	~ 6.8-7.0
10 mM Ammonium acetate + 0.025% Ammonia (25%)	~ 8.8-9.0

D- Organic Solvents:

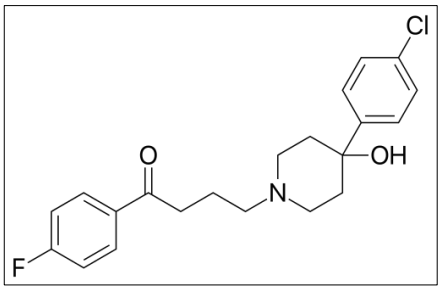
Solvent	%
Acetonitrile	100%
Methanol	100%
Acetonitrile: Methanol	50:50 % v/v

4.0 RESULTS AND DISCUSSION

4.1 Haloperidol

4.1.1: Structure of Haloperidol

Figure 4 : Structure of Haloperidol



Haloperidol is a typical or first order generation antipsychotic class of medicine used to treat psychosis and other mental and emotional conditions. It is chemically, 4-[4-(4-chlorophenyl)-4-hydroxypiperidin-1-yl]-1-(4-fluorophenyl) butan-1-one. Haloperidol is a phenylbutylpiperadine derivative with antipsychotic, neuroleptic, and antiemetic activities. Haloperidol is a psychotropic agent indicated for the treatment of schizophrenia. It also exerts sedative and antiemetic activity. Dopamine is a natural compound called a neurotransmitter and is involved in transmitting messages between brain cells. Haloperidol works by blocking a variety of receptors in the brain, particularly dopamine receptors. Haloperidol blocks the receptors that dopamine acts on and this prevents the over activity of dopamine in the brain. This helps to control psychotic illness.

4.1.2 Forced degradation study

To understand possible degradation of haloperidol, drug substance sample (1mg/mL) was exposed to different degradation conditions like acid, base, oxidation, and photolysis. These samples were tested using USP monograph method for Haloperidol drug substance.

Summary of the results observed after the exposure of drug to various stress conditions is depicted in Table 3.

Table 3 : Summary of forced degradation results of Haloperidol

Stress Condition	Experimental Condition	Temperature	Duration	Outcome
Thermal	NA	60°C	10 days	No degradation observed
Acidic	0.1N HCl, 5N HCl	55°C	7 days	No degradation observed
Alkaline	0.1N NaOH, 1N NaOH	55°C	3 Hrs.	One Impurity observed
Oxidation (H_2O_2)	30% V/V H2O2	Room Temperature	5 minutes	Two impurities observed
Photolytic	Keep in Photo stability chamber	Room Temperature	1.2 million lux hours & 200 Watt-hours/m2	Three impurities observed

1 Impurity was observed in base degradation, 2 impurities were observed in Oxidation and 3 impurities were observed in photolytic degradation conditions.

4.1.3 Stepwise UHPLC method development of Haloperidol

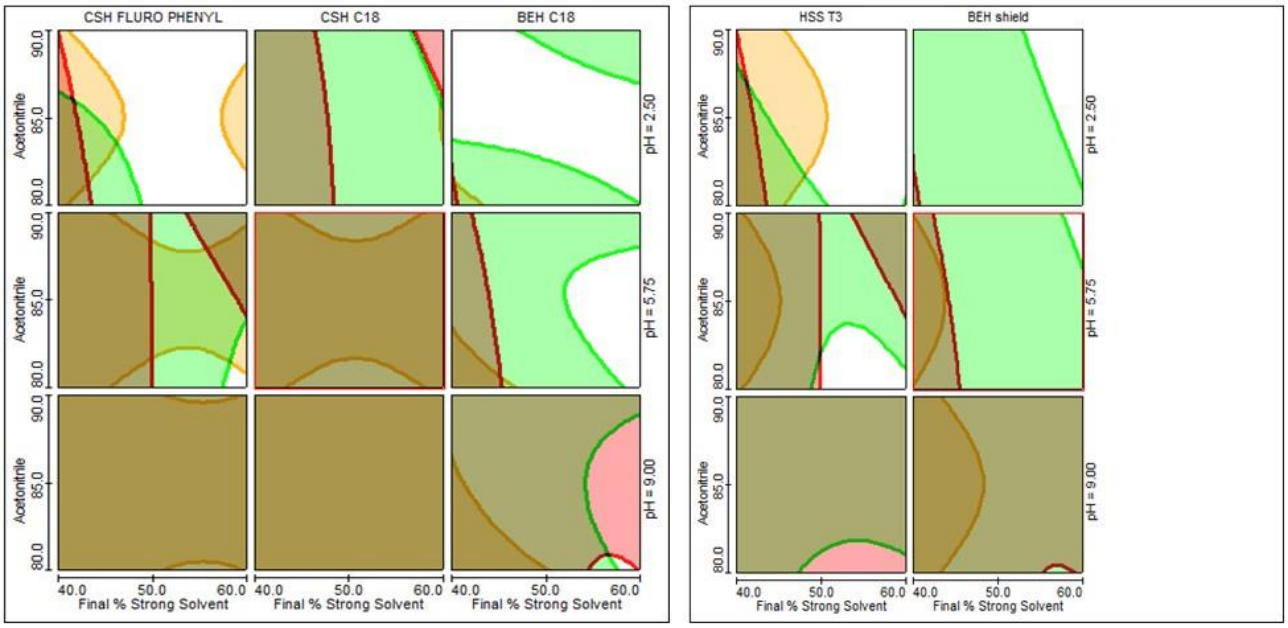
4.1.3.1 Sample Preparation: To generate worst case sample for DOE study, degradation samples of base, oxidation, and photolytic samples and about 1.0% of In-house available impurities Imp-A, Imp-B, Imp-C, Imp-D, Imp-E & Imp-F were mixed and diluted appropriately to get desired concentration of about 0.2mg/ml of Haloperidol. This solution contains total 13 peaks (1 active and 12 impurities).

4.1.3.2 Screening Methodology & Outcome: Screening was performed using 6 different column chemistries, 5 different mobile phase buffers covering PH range from 2.5 to 9.0 unit and Acetonitrile, Methanol and Mixture of Acetonitrile and Methanol (50:50) as organic solvents. Experiments were designed using Fusion AE method development software by S-matrix. Total 50 experiments were proposed by statistical design.

Screening Outcome: These 50 experiments data was evaluated for a) number of peaks (minimum 11 peaks), b) resolution > 2.0 & c) Tailing factor < 1.5 as CMA's (Critical Method Attributes). The results are evaluated based on the desirability factor (D). The best answer suggested by Fusion Software was, buffer pH 2.5, CSH Fluorophenyl column, 90 % Acetonitrile in organic modifier phase and first gradient organic modifier upto 48% with cumulative desirability factor 1.000. Trellis graph is showing results of experiments using different columns,

different pH, different % of Acetonitrile and % of final strong solvent.

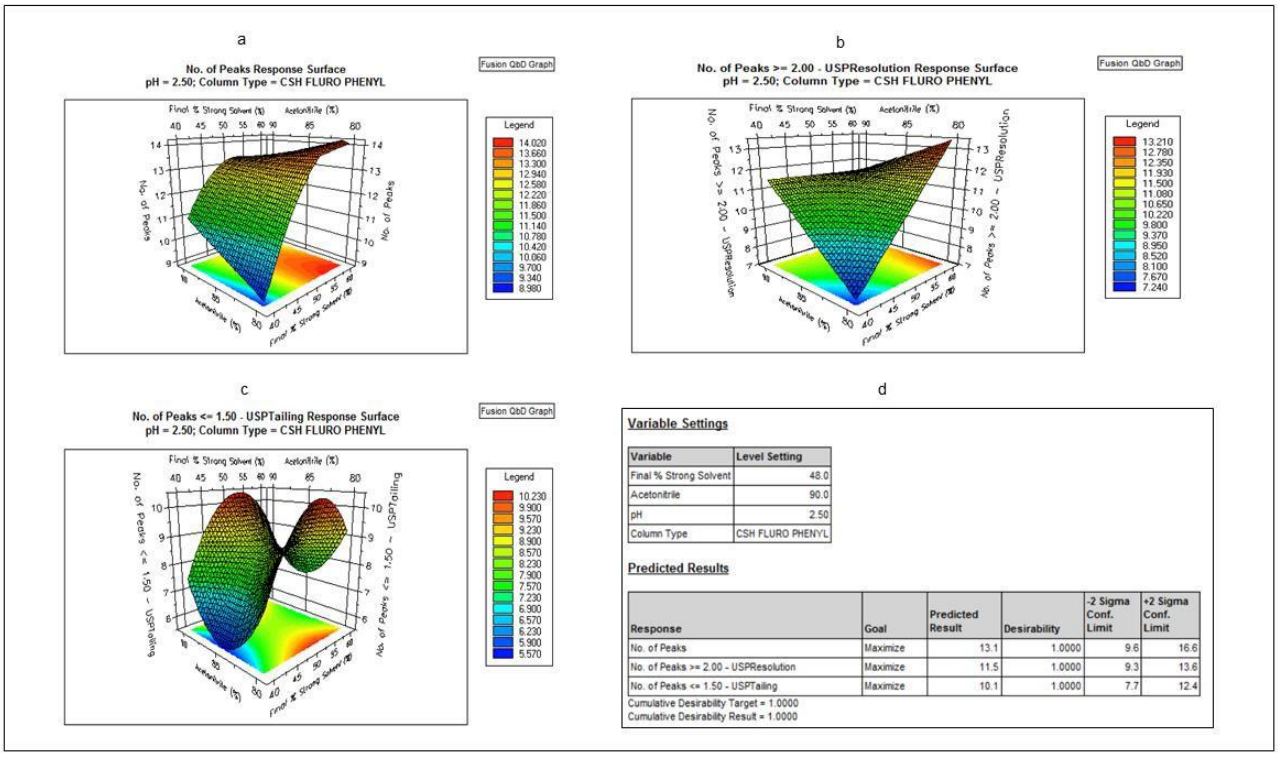
Figure 5 : Trellis graph for Screening experiment of Haloperidol



Colorless portion in the graphs shows the "Knowledge Space" the area where all the three parameters i.e., Number of peaks, Resolution between the peaks and Tailing factor results within expected range. CSH Fluorophenyl column at pH 2.5 showing good results as compared to other columns within the entire pH range of 2.5 to 9.0.

Multiple response series graphs (Figure 6) are showing the critical response outcome for CSH fluorophenyl column at pH 2.5. for number of peaks, number of peaks having resolution > 2 & number of peaks having tailing < 1.5.

Figure 6 : Multiple series graphs using pH 2.5 buffer and CSH Fluro phenyl column a) number of peaks b) number of peaks having resolution > = 2.00 c) number of peaks having tailing < = 1.5 d) Best answer suggested by Fusion software


a
No. of Peaks Response Surface
pH = 2.50; Column Type = CSH FLURO PHENYL
Fusion QbD Graph
Legend
b
No. of Peaks >= 2.00 - USPResolution Response Surface
pH = 2.50; Column Type = CSH FLURO PHENYL
c
No. of Peaks <= 1.50 - USPTailing Response Surface
pH = 2.50; Column Type = CSH FLURO PHENYL
d
Variable Settings
Predicted Results
Cumulative Desirability Target = 1.0000
Cumulative Desirability Result = 1.0000

Variable	Level Setting
Final % Strong Solvent	48.0
Acetonitrile	90.0
pH	2.50
Column Type	CSH FLURO PHENYL

Response	Goal	Predicted Result	Desirability	-2 Sigma Conf. Limit	+2 Sigma Conf. Limit
No. of Peaks	Maximize	13.1	1.0000	9.6	16.6
No. of Peaks >= 2.00 - USPResolution	Maximize	11.5	1.0000	9.3	13.6
No. of Peaks <= 1.50 - USPTailing	Maximize	10.1	1.0000	7.7	12.4

Based on the screening experiment Mobile phase buffer and column for further optimization were selected.

4.1.3.3 Optimization and Outcome:

Methodology: Based on screening experiments outcome (*Figure 5 & 6*), variables for further optimization were selected. Fine tuning of the flow rate, temperature, gradient, column length was performed to get the best possible separation between the peaks and fulfill the targeted CMA's.

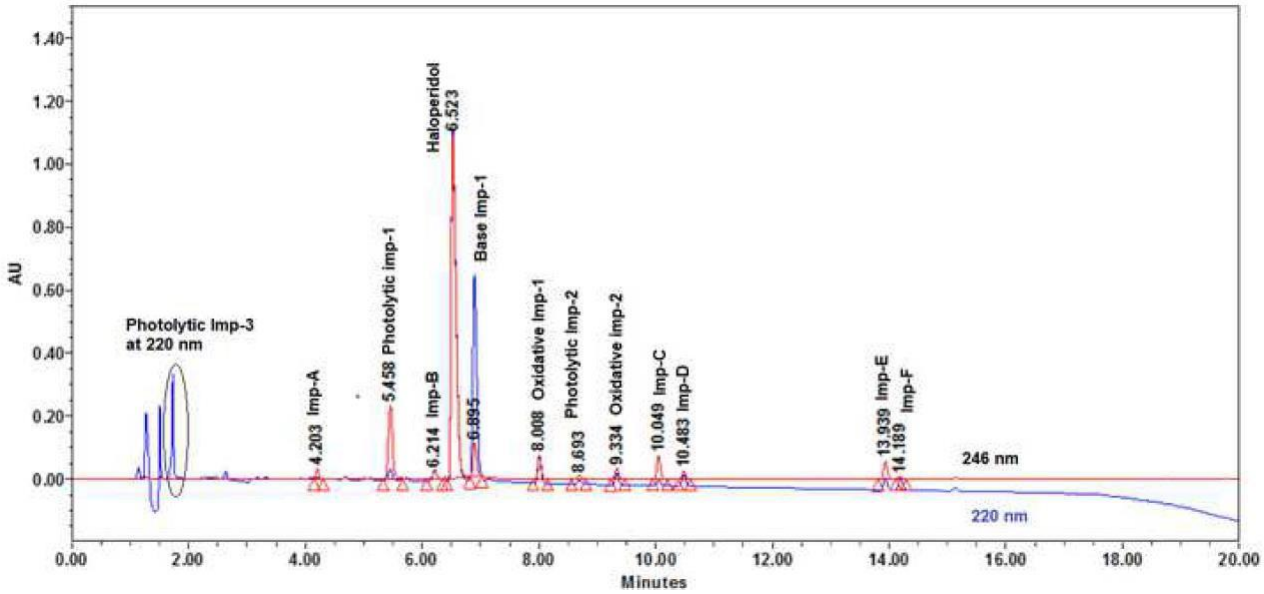
Outcome: Evaluation of optimization phase was done by evaluation of chromatograms visually and resolution between the peaks, tailing factor for active was checked. Based on the experiments the best separation was achieved by using following parameters:

Table 4 : Proposed method parameters based on optimization trials

UHPLC column	CSH Fluorophenyl column (150mm × 2.1 mm), 1.7µ
Mobile phase	10mM ammonium Acetate + 1ml TFA (A) and Acetonitrile: Methanol 80/20 v/v (B)
Flow rate	0.3 mL/ min
Column oven temperature	25°C
Injection volume	2µL
Wavelength	246 nm 220 nm (Photolytic Imp-1 & Imp F)
Concentration	200 µg/mL

Time (mins)	%A	%B
0	80	20
15	60	40
19	10	90
19.5	80	20
23	80	20

Figure 7 : Chromatogram: Spiked sample Chromatogram at 246 nm & 220 nm



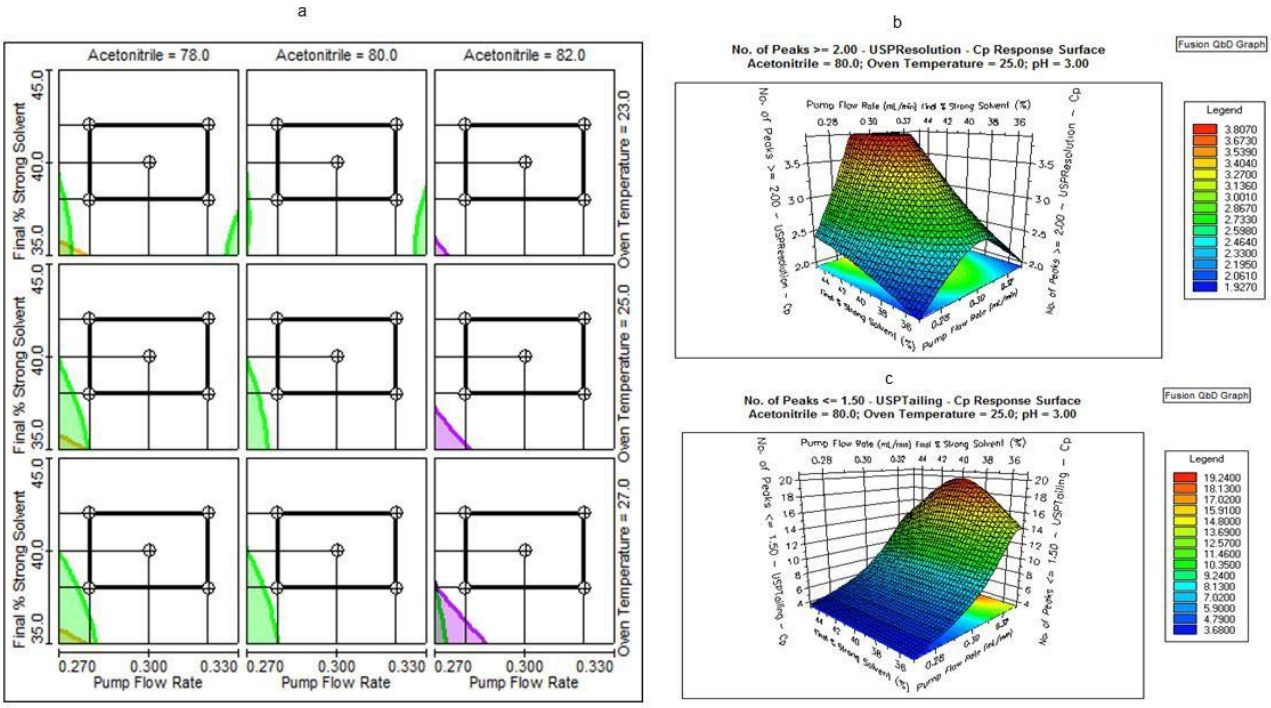
4.1.3.4 Robustness:

Robustness was divided into two parts 1) Chromatographic robustness 2) Sample preparation robustness

Chromatographic robustness:

During this study multiple variables were changed simultaneously, in different combinations using DOE approach. Four CMVs, Flow rate (± 0.03 ml), Concentration of ammonium acetate (± 2mM), % strong solvent in gradient step (± 5%) & column oven temperature (± 2°c) were changed simultaneously to evaluate the robustness of the method. A statistical process control metric, Capability index (Cp) is used to quantify and evaluate the impact of parameters variation in terms of critical quality and performance of the method. The design space (MODR) graph marked with Cp and PARs is shown in Figure 8. Total 13 peaks were separated, with 11 peaks having resolution > 2.0 and two peaks having resolution ≥ 1.5

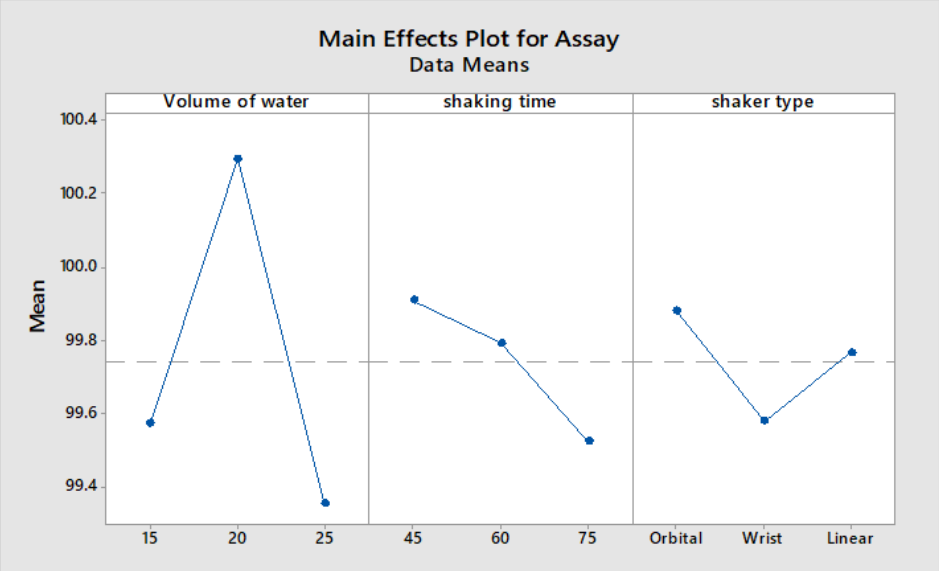
Figure 8 : Method Robustness outcome a) Design space and Proven Acceptable Ranges (PAR's) graph b) Multiple response graph for Resolution c) Multiple response graph for tailing factor.


a
Acetonitrile = 78.0
Acetonitrile = 80.0
Acetonitrile = 82.0
Final % Strong Solvent
Oven Temperature = 23.0
Oven Temperature = 25.0
Oven Temperature = 27.0
Pump Flow Rate
b
No. of Peaks >= 2.00 - USPResolution - Cp Response Surface
Acetonitrile = 80.0; Oven Temperature = 25.0; pH = 3.00
Fusion QbD Graph
Legend
c
No. of Peaks <= 1.50 - USPTailing - Cp Response Surface
Acetonitrile = 80.0; Oven Temperature = 25.0; pH = 3.00
Fusion QbD Graph
Legend

Sample Preparation Robustness:

To challenge the sample preparation procedure robustness for sample preparation was performed for Haloperidol 1 mg tablets by planning DOE experimental model using Minitab software. Full factorial design was created considering the Shaking time, amount of water in diluent & shaker type as CMV's for sample preparation step. Total of 27 sample preparations were done as per model suggested by Minitab and analyzed by using the optimized chromatographic conditions. Results generated were statistically evaluated using Minitab software. % Assay values found using combinations of variables are between 98.3 to 101.8 with % RSD of 0.9%.

Figure 9 : Sample preparation Robustness



4.1.4 UHPLC method validation Results of Haloperidol DS and Haloperidol 1 mg tablets

Method validation results for Haloperidol DS & DP are reported in Table 5.

Table 5: HPLC method validation results of Haloperidol

Parameters	Check points	Results
Specificity	- Resolution between all the impurities and Active peak. - For stress samples peak purity is passing for Haloperidol peak. PDA and Mass spectra of Haloperidol peak is matching at start, apex, and end.	Resolution > 1.5 Pass
Accuracy API -Assay Level (n =3 at each level) 80-100-120.0% w/w API- At lower level 0.05% /0.10* (*For DP) - 1.0% **Impurities** 0.05% -1.0%	- Recovery 98.0-102.0% - Recovery: 70.0-130.0% - Recovery: 70.0-130.0%	Pass Pass Pass
Linearity **API:** 0.05/0.10% -120.0% 80.0%-120.0% **Impurities:** 0.05/0.10% -1.0%	Correlation coefficient ≥ 0.995, Correlation coefficient ≥ 0.999 Graph: Linear Correlation coefficient ≥ 0.99 Graph: Linear	Pass Pass

Range: **API:** **0.05/0.10%-120.0%** **Impurities** **0.05/0.10 – 1.0% w/w**	Linearity, accuracy, and Precision is passing Linearity, accuracy, and Precision is passing	Pass Pass
Precision - Analysis repeatability (n= 6)	% RSD of assay active ≤ 2.0% % RSD for Impurity assay ≤ 10.0%	Pass Pass
LOD API & Impurities: 0.025%	S/N ≥ 3 Peak detectable	Pass
LOQ API & Impurities: 0.05%	S/N ≥ 10 Accuracy, linearity, and Precision is passing	Pass
System suitability	% RSD for 5 replicate injection of Reference solution Resolution between adjacent impurity and Haloperidol peak > 1.5	Pass (< 2.0%) Pass
Solution stability Checked at room temperature for 5 days	For API assay: Absolute Difference should not be more than 2.0% as compared to initial value. For Impurities: % Relative difference as compared to initial value should not be more than 20.0%	Pass Pass

4.1.5 Summary of Haloperidol

- One Impurity observed in base degradation, two impurities were observed in Oxidation and three impurities were observed in photolytic degradation conditions are separated from active and other impurities by using gradient UHPLC method.

- Stability indicating UHPLC method was successfully developed for assay of Haloperidol and related impurities in Haloperidol drug substance and drug products

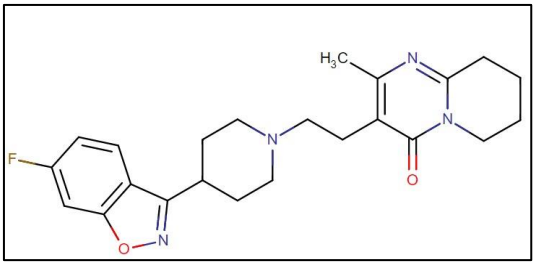
- Methods are mass compatible methods can be directly use for LC-MS investigation purpose.

- Effects of variables are known, if future if any executional issues found during method can be altered within design space and this change will not be considered as Method change.

4.2 Risperidone

4.2.1 : Structure of Risperidone

Figure 10 : Structure of Risperidone



Risperidone is a second-generation antipsychotic medication used in the treatment of mood and mental health conditions including schizophrenia and bipolar disorder. Risperidone is a legacy molecule which is approved in 1993 but still present in the WHO's list of essential Medicines. In 2018, it was the 159th most prescribed medication in the United States. Risperidone contains the functional groups of benzisoxazole and piperidine as part of its molecular structure. Peoples suffering from schizophrenia, bipolar disorder, and autism have an imbalance of certain neurotransmitters. Dopamine and serotonin are neurotransmitters known to be involved in regulating mood and behavior. Psychotic illness is considered to be caused by disturbances in the activity of neurotransmitters (mainly dopamine) in the brain. Risperidone works in the brain, where it affects various neurotransmitters, in particular dopamine and serotonin (5HT). Risperidone works by blocking the receptors in the brain that dopamine acts on. This prevents the excessive activity of dopamine.

4.2.2 Forced degradation studies of Risperidone

Risperidone drug substance was exposed to different stress conditions to understand the possible degradation of Risperidone molecule. Stress samples were analyzed by using USP monograph method for Risperidone Chromatographic Purity. Risperidone, drug substance sample (1mg/mL) was exposed to different degradation conditions like acid, base, oxidation, and photolysis.

Summary of the results observed after the exposure of drug to various stress conditions is depicted in Table 6.

Table 6 : Forced degradation data for Risperidone

Stress Condition	Experimental Condition	Temperature	Duration	Outcome
Thermal	NA	60°C	10 days	No degradation observed
Acidic	1N HCl, 5N HCl	55°C	7 days	No degradation observed
Alkaline	1N NaOH, 5N NaOH	55°C	3 Hrs.	No degradation observed
Oxidation (H_2O_2)	0.3, 3.0 % V/V H2O2	Room Temperature	7 days	One impurity observed
Photolytic	Keep in Photo stability chamber	Room Temperature	1.2 million lux hours & 200 Watt-hours/m2	No degradation observed

In stress study only 1 impurity was observed in oxidative degradation study.

4.2.3 UHPLC method development for Risperidone

4231 : Sample Preparation: For DOE study Sample was prepared by mixing Risperidone drug substance at 0.5 mg/mL and available impurities at 0.005mg/mL level in mixture of Water: Methanol in the ratio of 10:90 v/v. This solution contains total 9 peaks (1 active and 8 impurities).

4232 : Screening and outcome: The main aim of the Screening phase is to identify the suitable column and ideal pH range for the separation of compound and related impurities. For screening experiments 5 different column chemistries were selected based on bonding, pH range and selectivity. HSS C18, BEH C18, CSH C18, BEH Phenyl and CSH Fluro Phenyl columns were used [18]. Screening of the mobile phase Buffer pH was studied in the pH range of 2.5 to 9.0 unit. Flow rate and column temperature was kept constant. Gradient program used during screening Phases Time/Mobile phase B, T0/10, T10-25/70, T12-27/80, T32/10. Experiments were designed using Fusion AE method development software by S-matrix. Total 53 experiments were proposed by statistical design. G-efficiency computed value for the experimental plan was 59.1 (Limit > 50%) and average predicted variance was 29.1 (limit < 50). It shows the experimental design created is within design optimality statistics limits. After running these 53 experiments data was evaluated for critical method attributes as a) number of peaks b) Number of peaks having resolution ≥ 2.0 and peak result base response like c) Tailing factor ≤ 1.2 & d) Maximum resolution between impurity and Risperidone peak.

Outcome of Screening: Best possible answer suggested by the Fusion software was as given below.

Answer #1

Variable Settings

Variable	Level Setting
Gradient Time	24.14
Acetonitrile	64.0
pH	8.00
Column Type	HSS

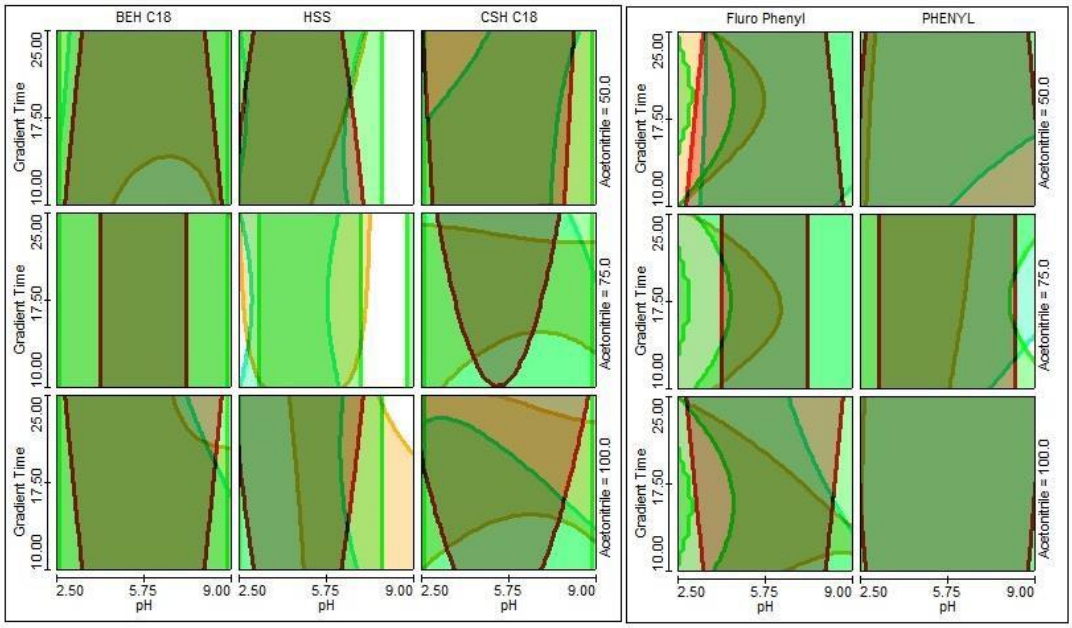
Predicted Results

Response	Goal	Predicted Result	Desirability	-2 Sigma Conf. Limit	+2 Sigma Conf. Limit
No. of Peaks	Maximize	10.0	0.9874	8.3	11.6
No. of Peaks <= 1.20 - USPTailing	Maximize	8.7	1.0000	7.9	8.7
Max Peak #1 - USPResolution	Maximize	3.00	1.0000	2.04	4.02
No. of Peaks >= 2.00 - USPResolution	Maximize	10.8	1.0000	9.5	12.1

Cumulative Desirability Target = 1.0000
Cumulative Desirability Result = 0.9968

Trellis graph is showing results of experiments using different columns, different pH, different % of Acetonitrile, and different gradient time.

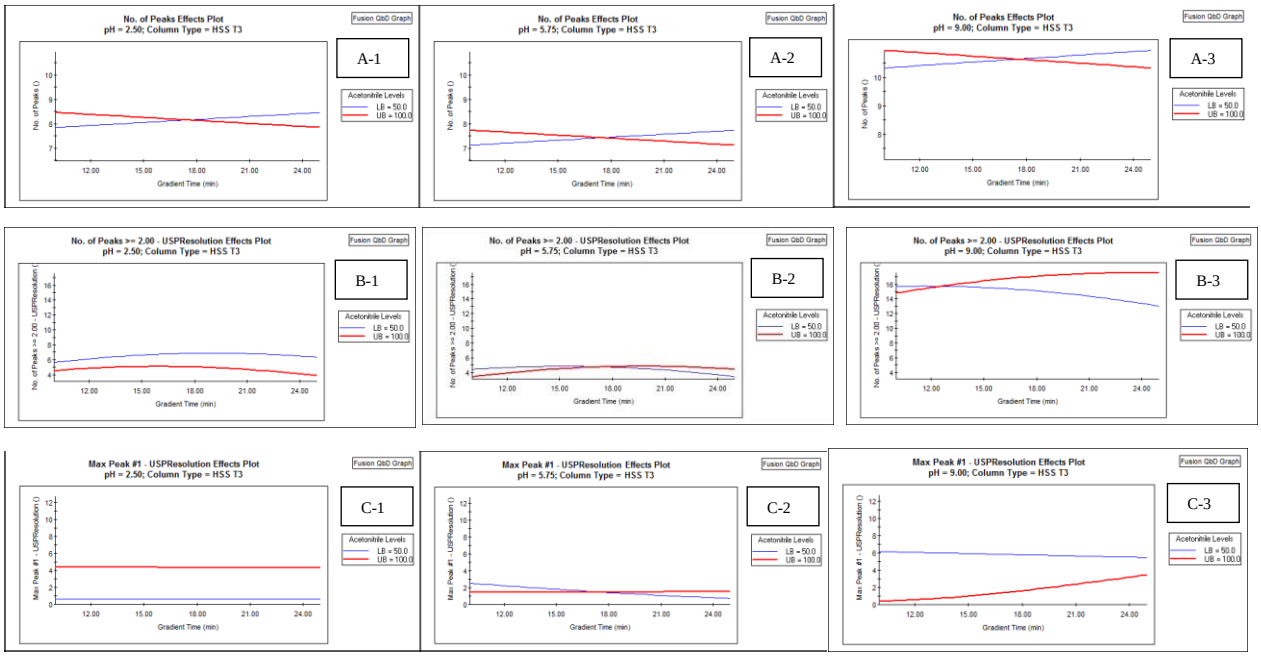
Figure 11 : Trellis graph for screening experiments



Colorless portion shown in the above trellis graph shows the most suitable conditions to achieve the favorable results for CMA (Critical method attributes). Based on the trellis graph it was found that only HSS column at basic pH range showing positive outcome. For separation of Risperidone and related impurities HSS column and buffer with basic pH are suitable.

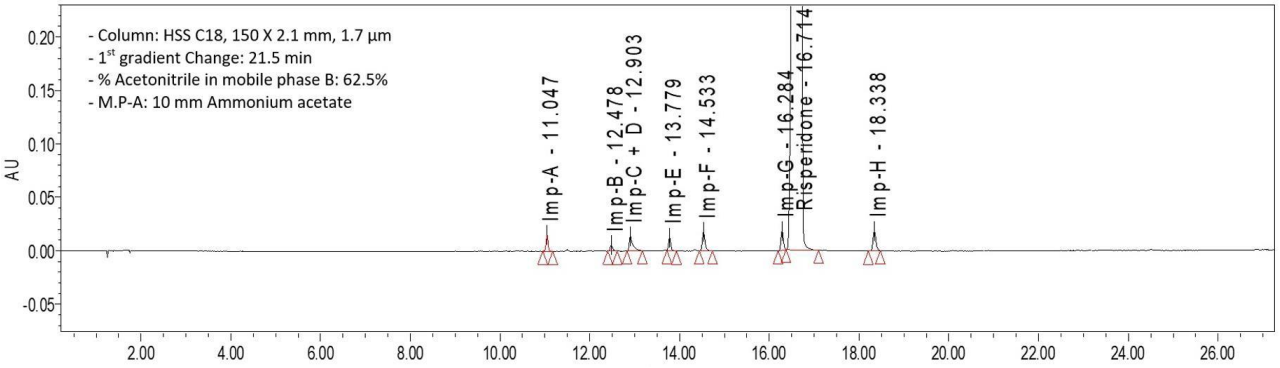
Effects of Critical method variables (CMV's) on CMA's (critical method attributes) is shown in Figure 12. A1-A3) For number of peaks, with the increase in gradient time, number of peaks detected decreases if % of Acetonitrile in Mobile phase B is increased. B1-B3) For number of peaks having resolution more than 2.0, with the increase in gradient time, number of peaks having resolution more than 2.0 increases with the increase in % of acetonitrile in mobile phase B. C1-C3) For resolution between Risperidone and adjacent impurity peak, with the increase in gradient time resolution between Risperidone and adjacent impurity peak either remain unchanged at lower pH and increase at the higher pH when % of Acetonitrile in Mobile phase B is increased. This information is very useful information for further optimization of method.

Figure 12 : Effect plots for HSS C18 column showing correlation between the variables


No. of Peaks Effects Plot
pH = 2.50; Column Type = HSS T3
A-1
No. of Peaks Effects Plot
pH = 5.75; Column Type = HSS T3
A-2
No. of Peaks Effects Plot
pH = 9.00; Column Type = HSS T3
A-3
No. of Peaks >= 2.00 - USPResolution Effects Plot
pH = 2.50; Column Type = HSS T3
B-1
No. of Peaks >= 2.00 - USPResolution Effects Plot
pH = 5.75; Column Type = HSS T3
B-2
No. of Peaks >= 2.00 - USPResolution Effects Plot
pH = 9.00; Column Type = HSS T3
B-3
Max Peak #1 - USPResolution Effects Plot
pH = 2.50; Column Type = HSS T3
C-1
Max Peak #1 - USPResolution Effects Plot
pH = 5.75; Column Type = HSS T3
C-2
Max Peak #1 - USPResolution Effects Plot
pH = 9.00; Column Type = HSS T3
C-3
Gradient Time (min)
Acetonitrile Levels
LB = 50.0
UB = 100.0

For HSS column pH operating range is upto pH 8.0. No experimental data at pH 8.0 is available. Data close to pH 8 was pH 6.8 buffer (10 mm Ammonium acetate buffer) was evaluated. Chromatograms using pH 6.8 buffer & HSS column is given in figure 2. This trial is close to the experimental conditions predicted by the Fusion software.

Figure 13 : Chromatogram obtained for Screening experiment


- Column: HSS C18, 150 X 2.1 mm, 1.7 µm
- 1st gradient Change: 21.5 min
- % Acetonitrile in mobile phase B: 62.5%
- M.P-A: 10 mm Ammonium acetate
AU
Imp-A - 11.047
Imp-B - 12.478
Imp-C + D - 12.903
Imp-E - 13.779
Imp-F - 14.533
Imp-G - 16.284
Risperidone - 16.714
Imp-H - 18.338

Chromatogram shows that except impurity C & D all other impurities are separated. Based on the screening experiment outcome HSS column and pH 6.8 buffer are selected for further optimization phase.

4.2.3.4: Optimization & Outcome:

Based on the screening outcome critical variables for further optimization were selected. It was observed that % Acetonitrile and gradient time is playing critical role in the separation of the peaks. Second screening or optimization initiated with the evaluation of impact of 100% Methanol. HSS C18 150 X 2.1 mm, 1.7 µm column and mobile phase pH 6.8 and 100% Methanol as mobile phase B was used for further screening. Optimization/second screening was done specifically targeting the reduction in the run time with optimum separation of peaks. Further optimization study was performed using Fusion software and using Column thermostat (25 °C – 45°C), Flow rate (0.25 mL to 0.35 mL), Initial gradient composition (Initial % Organic: 10.0%-30.0%) as variables. Optimization trials were performed using 100 mm HSS C18 column and 10 mM Ammonium acetate buffer. Fine tuning of the flow rate, temperature, initial gradient composition was performed to get the best possible separation between the peaks and fulfill the targeted CMA's.

Outcome of Optimization: Using 100% methanol and HSS C18 Column of 150 mm length all the peaks are well separated (Figure 14). During optimization the main aim was to reduce the run time

with optimum separation. 50 mm and 100 mm column length HSS C18 columns of 2.1 mm id and 1.7 µm particle size were studied with some modifications in gradient. For 50 mm and 100 mm column peaks were separated with short run time but optimum separation was achieved on 100 mm HSS C18 column with run time of about approximately 12.0 minutes. During optimization study the CMA's considered are a) Number of peaks having resolution > 1.5 and > 2.0, b) USP Plate count for Risperidone peak > 15000, C) Resolution between the Risperidone and peak eluting closer to Risperidone peak > 1.5. Pump flow rates were studied between 0.25 mL/min to 0.35 mL/min, column temperature was studied between (38°C–45°C) and Initial % organic in gradient was studied in the range of 10.0%. to 30.0%. Evaluation of optimization phase was done by Fusion software.

Figure 14 : Chromatogram obtained with optimized conditions

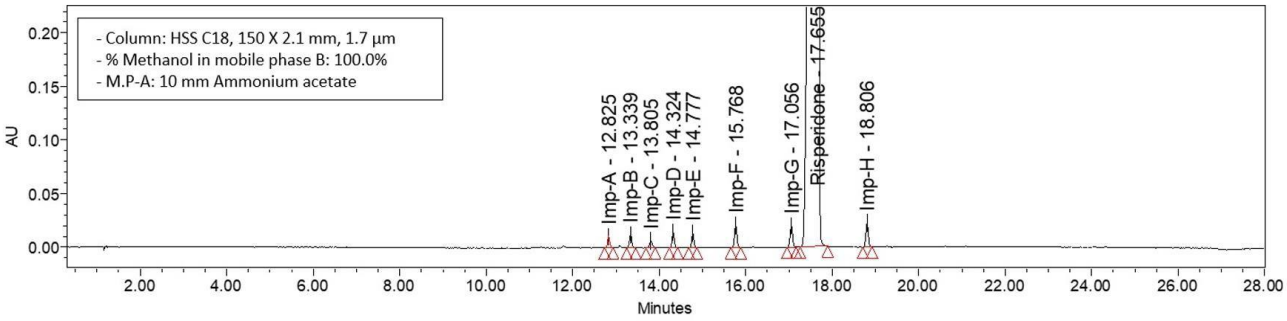


Figure 16 shows the graph for combined impact of variables on the critical method attributes. White or unshaded portion of the graph shows the region where all the CMA's are passing the predefined acceptance criteria's at different variable conditions. Design space or MODR-Method Operable design region is shown by white color and PAR: "Proven Acceptable Region" shown by the square and Target Point "T". Pump Flow rate of 0.30 ml, % Initial organic at 20%. Column temperature in the range of 38°C to 45°C and Gradient program used as follows Time/Mobile phase B, T0/20, T6.71/85, T9.71/85, T10.2/20, T12.0/20. The optimized method chromatogram showing separation between peaks is shown in figure 15.

Figure 15 : Chromatogram with optimized parameters using 100 mm column

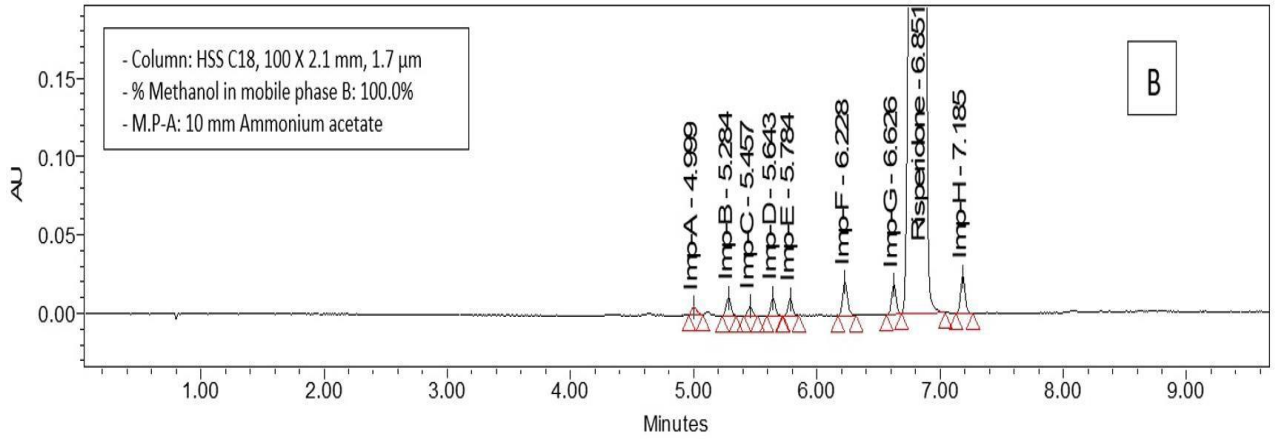


Figure 16 : Trellis graph for Optimization conditions showing impact combined impact of variables on the critical method attributes

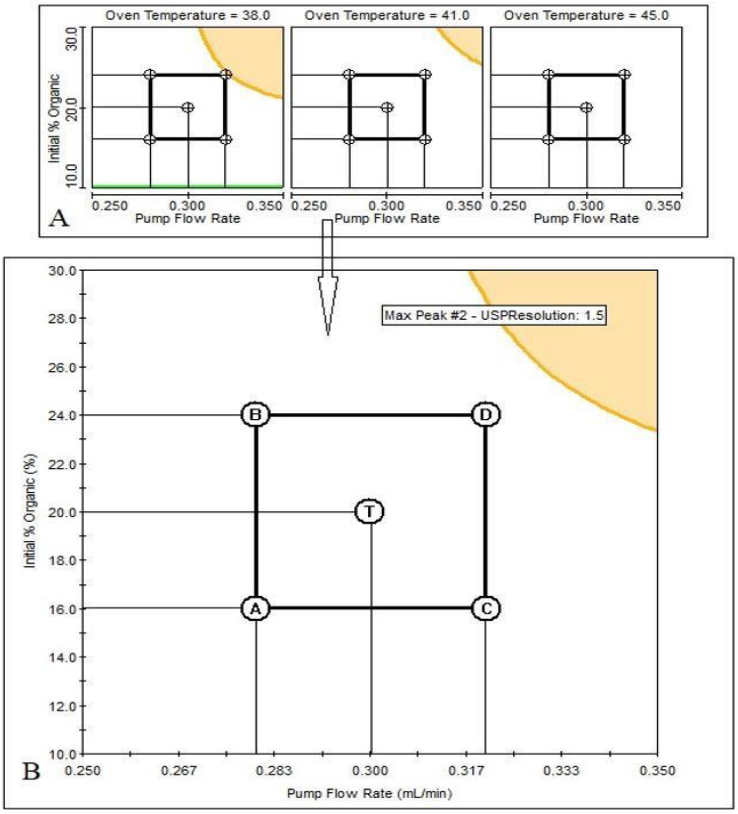
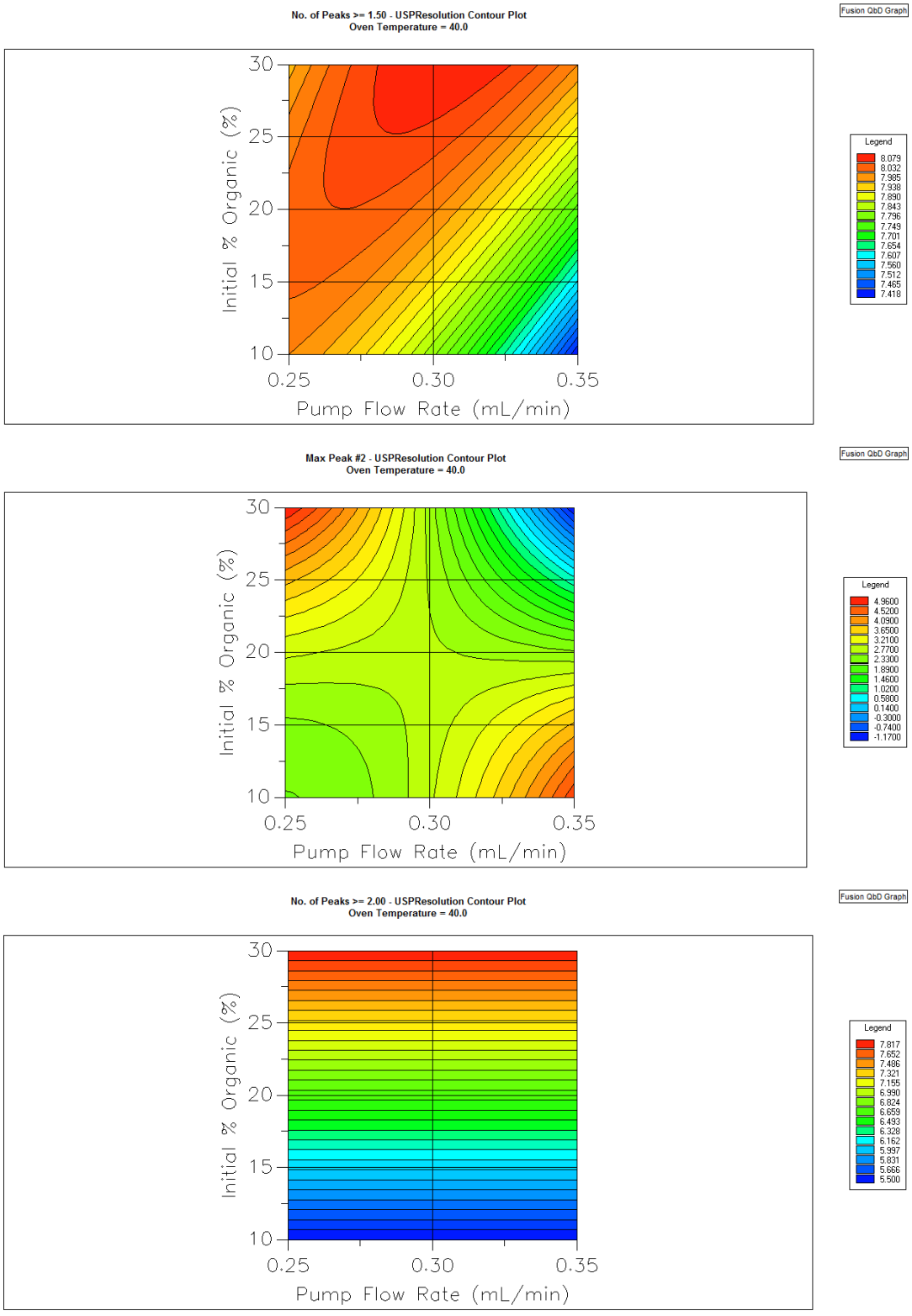
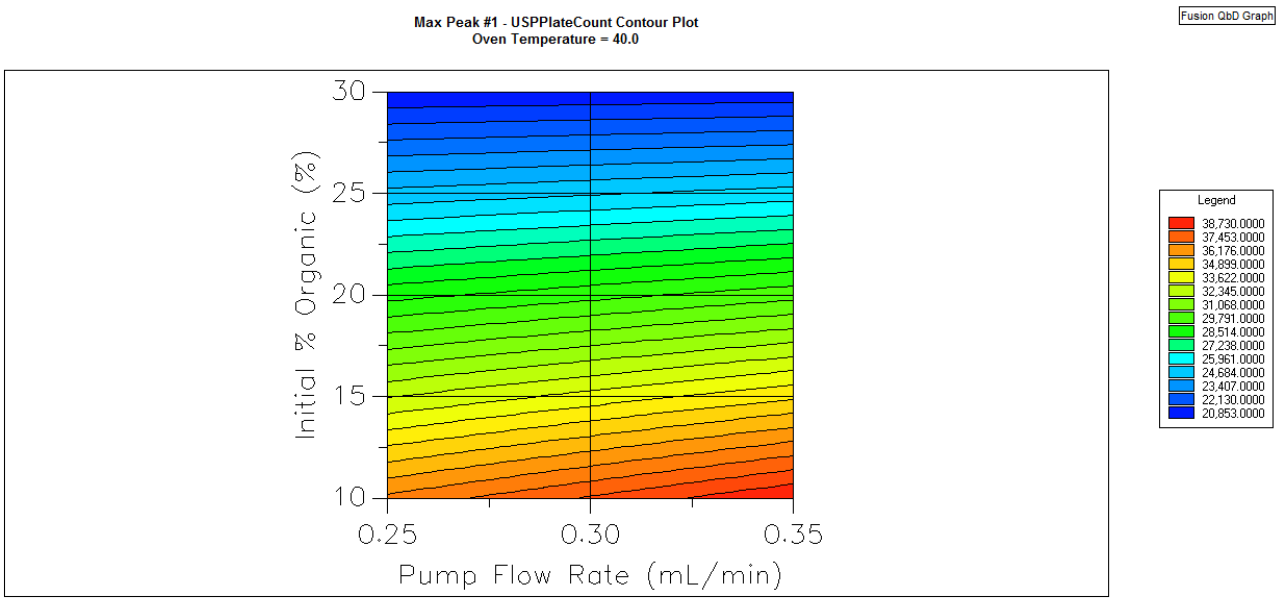


Figure 17 : Contour plots showing impact of % organic in the gradient start and column oven temperature.





In contour plots red color is showing the area where best possible results are achieved. This is the area where positive results obtained for CMA's. Blue shaded area shows that conditions where lowest results are achieved.

Based on the optimization outcome proposed method parameters are as follows,

Table 7 : Optimized method parameters for Risperidone

Column:	HSS C18, 100 mm length × 2.1 mm i.d., 1.7 µm particle size.	
Column Temperature:	40 °C	
Auto-Sampler Temperature:	Ambient	
Flow Rate:	0.3 mL/min	
Detection:	UV	
Wavelength:	276 nm	
Injection Volume:	3 µL	
Elution Mode:	Mobile Phase A: 10 mM (± 2 mM) Ammonium acetate Mobile Phase B: Methanol	
Time in Min (min)	A (% vol)	B (% vol)
0	80	20
6.51	15	85
9.0	15	85
9.2	80	20
12.0	80	20

Further method was challenged through robustness study.

4.2.3.4: Method Robustness and outcome:

The robustness of a given method is defined as its inherent ability to perform with the required precision in normal use. In other words, to perform consistently given the expected random variations in the experiment variables about their setpoints that will occur in normal use over time. In case of method developments by QBD separate robustness study is not required. MODR region identified in optimization phase can be considered as Robust region and any changes made within this range cannot be considered as any change in the method. However separate robustness study was performed to challenge the method with higher temperature variation and buffer concentration. In traditional robustness study normally, developer change the one parameter at a time by keeping pother parameters constant, but during this study multiple variables were changed simultaneously, in different combinations using DOE approach. Four CMVs, Flow rate (± 0.05 mL), Concentration of ammonium acetate (± 2mM), % strong solvent in Initial gradient (± 10.0%) & column oven temperature (± 5°C) were changed simultaneously to evaluate the robustness of the method.

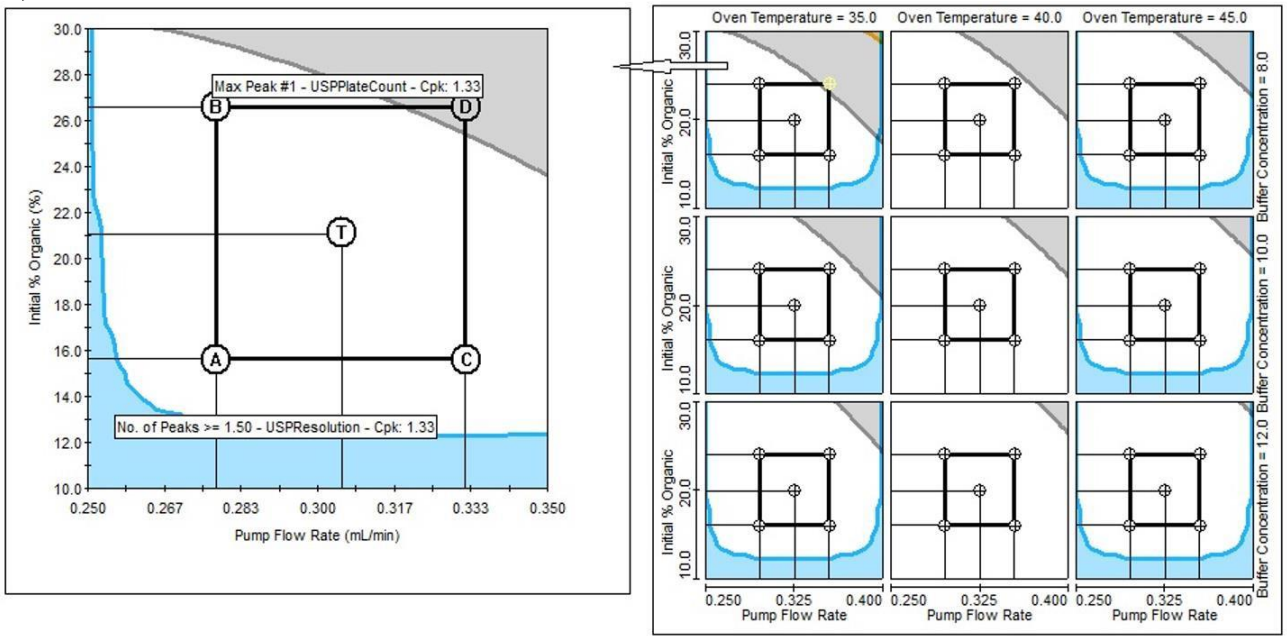
Outcome of Robustness study:

During evaluation of robustness during this study Cpk is used as robustness capability metrics. Limit for Cpk value is set as Cpk > 1.33. For robustness evaluation lower specification limits were considered for the resolution between the peaks > 1.5, Maximum resolution between Risperidone and adjacent peak > 1.5 and USP plates for the Risperidone peaks > 15,000. The design space (MODR) graph and PARs is shown in Figure 18. In figure 18 white surface shows design space & squares shows the PAR's. Based on the evaluation of the figure 18 it was found that with mobile phase concentration of 8 mM and column temperature 35°C the grey shaded area which represents USP plate count is coming towards the PAR region, which means lowering buffer concentration and column temperature may impact USP plate count. Single graph was studied, and it was found that using Mobile phase buffer 8 mM and column temperature 35°C at higher flow rate and higher % of organic the Cpk value for Plate count is < 1.33, which shows USP Plate count criteria of > 15000 is not achieved when these parameters are used which is shown by grey shaded area is touching the Point D of PAR region as shown in figure 18-A. Data was evaluated with use of 9 mM ammonium acetate keeping all other parameters same and found all the CMA's are achieving the goal and Cpk value > 1.33 (Figure 5). However, based on the figure 18-B it is clear that there is no impact of temperature, Buffer concentration and % of acetonitrile in the MODR region and all the CMA's are within acceptance criteria. Cpk response graphs for resolution and USP Plate count using control condition method parameters is given in online resource 7 and it shows that Cpk values are > 1.33 that means

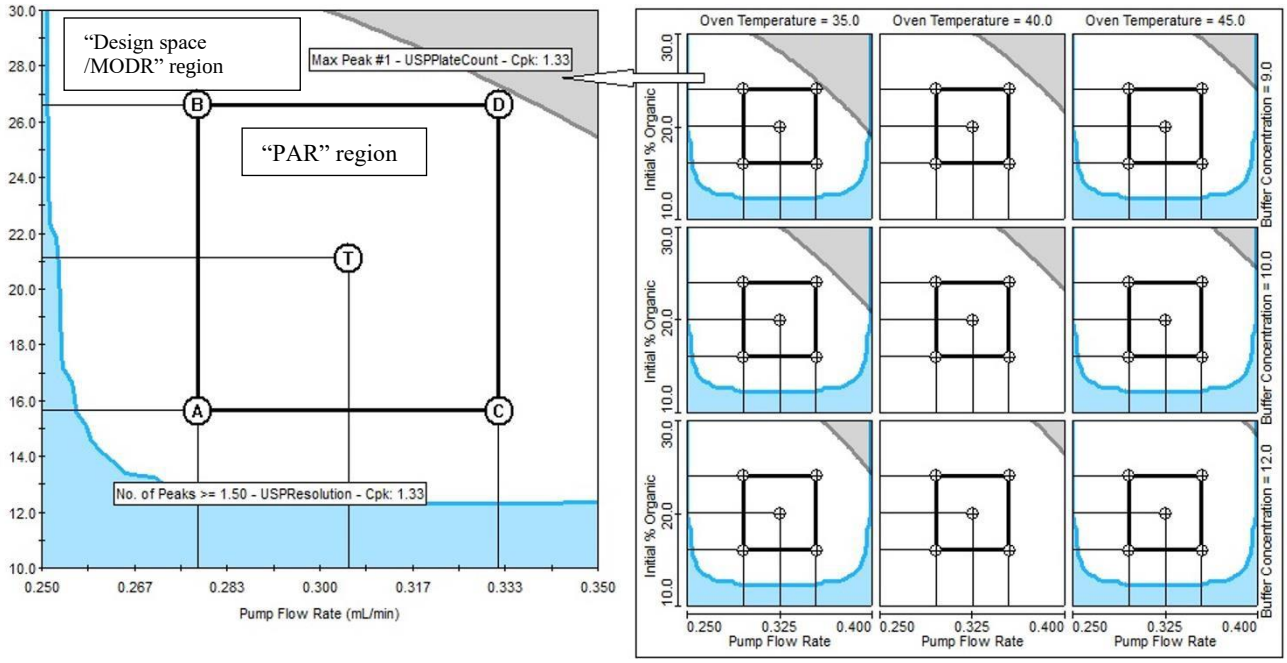
USP resolution and plate count criteria are passing. The experimental response values were founding to be within confidence limit. Total 9 peaks were separated, with resolution > 1.5 and USP Plate count for Risperidone > 10,000.

Figure 18 : Trellis graph for Robustness study

A)


Max Peak #1 - USPPlateCount - Cpk: 1.33
No. of Peaks >= 1.50 - USPResolution - Cpk: 1.33
Initial % Organic (%)
Pump Flow Rate (mL/min)
Oven Temperature = 35.0
Oven Temperature = 40.0
Oven Temperature = 45.0
Initial % Organic
Pump Flow Rate
Buffer Concentration = 8.0
Buffer Concentration = 10.0
Buffer Concentration = 12.0

B)


"Design space /MODR" region
"PAR" region
Max Peak #1 - USPPlateCount - Cpk: 1.33
No. of Peaks >= 1.50 - USPResolution - Cpk: 1.33
Pump Flow Rate (mL/min)
Oven Temperature = 35.0
Oven Temperature = 40.0
Oven Temperature = 45.0
Initial % Organic
Pump Flow Rate
Buffer Concentration = 9.0
Buffer Concentration = 10.0
Buffer Concentration = 12.0

4.2.4 Analytical Method Validation

To prove method is suitable for intended use and accurate and precise, analytical method validation was performed according to ICH Q2 (R1) [19] & ANVISA guidelines (RDC N0 166, Dated July 24th, 2017) [20]. Specificity, Accuracy, Precision (System & analysis repeatability), Linearity, Limit of Detection, Limit of Quantification was performed.

Table 8 : UHPLC method validation results of Risperidone

Parameters	Check points	Results
Specificity	- Resolution between all the impurities and Active peak. - For stress samples peak purity is passing for Risperidone peak. PDA and Mass spectra of Risperidone peak is matching at start, apex and end.	Resolution > 1.5 Pass
Accuracy API -Assay Level (n =3 at each level) 80-100-120.0% w/w API- At lower level 0.05% -1.0% **Impurities** 0.05% -1.0%	- Recovery 98.0-102.0% - Recovery: 70.0-130.0% - Recovery: 70.0-130.0%	Pass Pass Pass
Linearity **API:** 0.05/0.10% -120.0% 80.0%-120.0% **Impurities:** 0.05/0.10% -1.0%	Correlation coefficient ≥ 0.995, Correlation coefficient ≥ 0.999 Graph: Linear Correlation coefficient ≥ 0.99 Graph: Linear	Pass Pass

Range: **<u>API:</u>** **0.05/0.10%-120.0%** **<u>Impurities</u>** **0.05/0.10 – 1.0% w/w**	Linearity, accuracy and Precision is passing Linearity, accuracy, and Precision is passing	Pass Pass
Precision - Analysis repeatability (n= 6)	% RSD of assay active ≤ 2.0% % RSD for Impurity assay ≤ 10.0%	Pass Pass
LOD API & Impurities: 0.025%	S/N ≥ 3 Peak detectable	Pass
LOQ API & Impurities: 0.05%	S/N ≥ 10 Accuracy, linearity, and Precision is passing	Pass
System suitability	% RSD for 5 replicate injection of Reference solution Resolution between adjacent impurity and Haloperidol peak > 1.5	Pass (< 2.0%) Pass
Solution stability Checked at room temperature for 7 days	For API assay: Absolute Difference should not be more than 2.0% as compared to initial value. For Impurities: % Relative difference as compared to initial value should not be more than 20.0%	Pass Pass

Since all the acceptance criteria have been met the study demonstrates that the test method is suitable for its intended use.

4.2.5 Other Formulations:

Chromatographic parameters also checked for Risperdal 0.5,2,3,4 mg tablets and Risperidone 1.0 mg/ml oral solution.

A) Sample preparation for 0.5,2,3,4 mg tablets:

Transfer accurately number of tablets (0.5 mg: 20 units, 2 mg: 5 units, 3 mg: 4 tablets, 4 mg: 3 tablets) to 20 ml volumetric flask. Add about 2 ml of 10 mm ammonium acetate and disperse. Add about 10 ml of methanol and sonicate for about 15 minutes. Dilute to volume using methanol. Centrifuge the solution at 3000 RPM for about 30 minutes. Filter the supernatant solution through 0.2 µm GHP filter.

B) Sample preparation for Risperidone orals solution:

Using volumetric pipette transfer 10.0 ml of Risperdal oral solution to 20 ml volumetric flask. Add about 2 ml of 10 mm ammonium acetate and 5 ml of Methanol. Vortex for about 2 minutes. Dilute to volume using methanol. For Benzoic acid injection volume to be reduced to 2µL.

% Assay and analysis repeatability was checked for each formulation. Mean % assay and analysis repeatability results (expressed as % RSD) was reported.

Table 9 : Precision and Analysis repeatability results for Risperidone formulations

Formulation	Mean % Assay	% RSD (Analysis repeatability)
Risperidone 0.5 mg tablets	96.8	0.4
Risperidone 2 mg tablets	101.1	0.5
Risperidone 3 mg tablets	99.9	0.7
Risperidone 4 mg tablets	101.0	0.2
Risperidone 1mg/ml oral solution	100.3	0.2
Risperidone 1 mg/ml orals solution- Benzoic acid	99.1	07

Figure 19: Chromatogram: Assay /Purity of Risperidone in Risperidone 1mg/mL oral solution

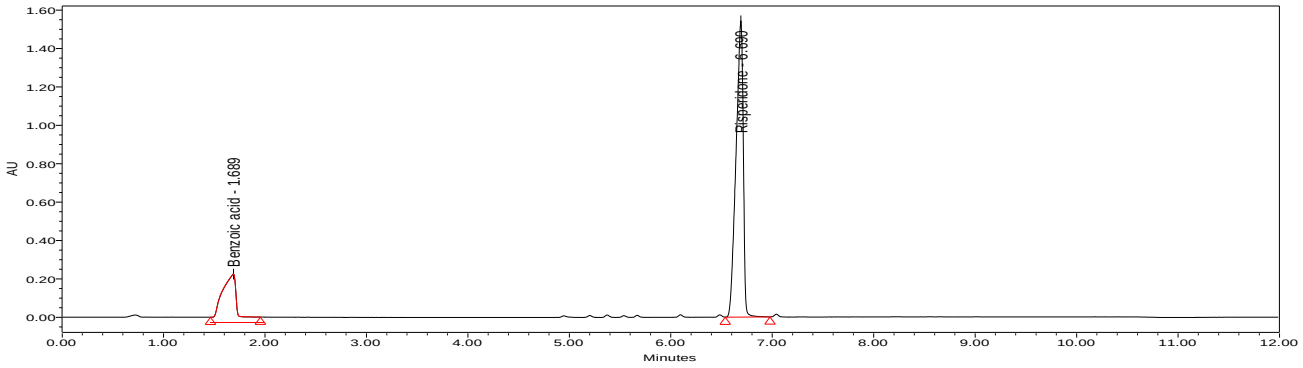


Figure 20 : Chromatogram: Assay of Benzoic acid in Risperidone 1mg/mL oral solution

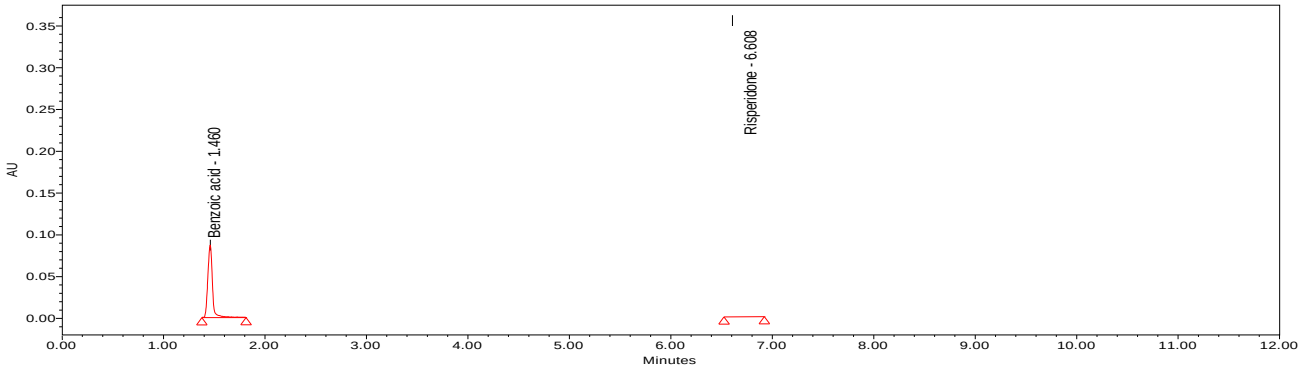
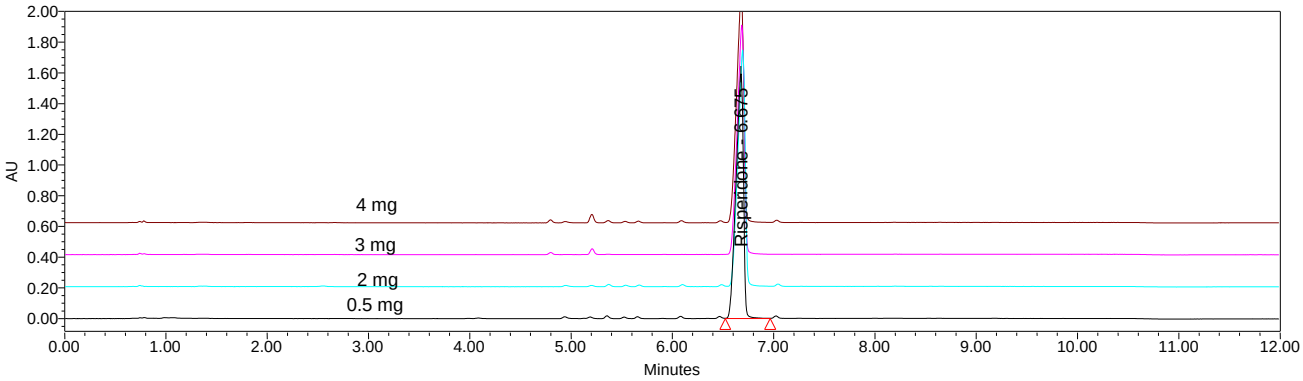


Figure 21 : Chromatogram: Assay /Purity of Risperidone in Risperidone Tablets (0.5, 2 , 3 & 4 mg).



Base on the method validation final method parameters are as follows,

Table 10 : Method Parameters for Risperidone DS & DP

Column:	HSS C18, 100 mm length × 2.1 mm i.d., 1.7 µm particle size.	
Column Temperature:	40 °C (± 2 °C)	
Auto-Sampler Temperature:	Ambient	
Flow Rate:	0.3 mL/min (± 0.02 mL)	
Detection:	UV	
Wavelength:	276 nm	
Injection Volume:	3 µL (2 µL for Benzoic acid)	
Elution Mode:	Mobile Phase A: 10 mM (± 2 mM) Ammonium acetate Mobile Phase B: Methanol	
Time in Min	A (% vol)	B (% vol)
0	80	20
6.51	15	85
9.0	15	85
9.2	80	20
12.0	80	20

4.2.6 Summary of Risperidone

- One Impurity observed in Oxidative degradation. All the impurities are separated from active and each other by using gradient UHPLC method.
- Stability indicating UHPLC method was successfully developed for assay of Risperidone and related impurities in Risperidone drug substance and drug products
- Methods are mass compatible methods can be directly use for LC-MS investigation purpose.
- Effects of variables are known, if future if any executional issues found during method can be altered within design space and this change will not be considered as Method change.

5 Summary and conclusion

- Stability indicating UHPLC methods were successfully developed for Haloperidol & Risperidone drug substance and drug products
- Same method can be applied starting from development of molecule to complete shelf-life drug product or drug substance.
- As method was developed using degraded samples, method is capable to separate if any unknown impurities observed during stability testing.
- Methods are mass compatible methods can be directly use for LC-MS investigation purpose.
- Effects of variables are known, if future if any executional issues found during method can be altered within design space and this change will not be considered as Method change.
- The proposed analytical methods can be applied for routine quality control analysis of stability samples.

➢ List of Publications

1) A. Pawar, N. Pandita, Statistically Designed, Targeted Profile UPLC Method Development for Assay and Purity of Haloperidol in Haloperidol Drug Substance and Haloperidol 1 mg Tablets, Chromatographia 83(6) (2020) 725-737.

2) Application of "Method Operable Design Region" (MODR) approach for the development of UHPLC method for assay and purity of Risperidone in Risperidone drug substance and other formulations: Under Review – Biomedical Chromatography, BMC-22-0211

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